Reference: *Biol. Bull.* 157: 536–547. (December, 1979)

IRREVERSIBLE NONGENETIC TEMPERATURE ADAPTATION OF OXYGEN UPTAKE IN CLONES OF THE SEA ANEMONE *HALIPLANELLA LUCIAE* (VERRILL)

WILLIAM E. ZAMER AND CHARLOTTE P. MANGUM

Department of Biology, College of William and Mary, Williamsburg, Virginia 23185

For many years physiological differences have been observed among latitudinally separated populations of the same species (Bullock, 1955; Prosser, 1955; Vernberg, 1962). These differences have been attributed to a variety of phenotypic adaptations and also to genetic variation.

One of the earliest examples of physiological variation was the difference in acutely measured bell pulsation rates of the jellyfish *Aurelia aurita* collected from Nova Scotia and Florida (Mayer, 1914), the direction of which agreed with Krogh's (1916) prediction of increased rates in cold-adapted animals compared to warm-adapted animals, when the rates are measured at a common temperature. More recent studies of oxygen uptake as well as motor activity in cnidarians confirmed the existence of distinct responses in latitudinally separated populations within a species (Sassaman and Mangum, 1970; Mangum, Oakes, and Shick, 1972; Shick, 1976).

In many examples of intraspecific variation, latitudinal differences disappear with acclimation to common conditions, indicating their phenotypic origin. In several studies of latitudinally separated populations however, the differences persist despite prolonged exposure to common conditions, which has been interpreted alternatively as genetic divergence and "irreversible nongenetic adaption" (Kinne, 1962). There are numerous examples of variation which is highly likely to have a genetic basis, *e.g.* interspecific variation (Vernberg, 1962). The possibility of nongenetic but irreversible adaptation within species is less certain.

Evidence of this phenomenon in *Drosophila subobscura* was presented by Smith (1956), who induced variation in heat tolerance among groups of flies raised at different temperatures. The differences, which did not disappear completely upon acclimation to a common temperature, were adaptive in character; flies developing at the higher temperature survived longer at an upper lethal limit. However, selection, operating during development, could conceivably explain these results. Kinne (1962) reported that fish developed from eggs which had been transferred to a different salinity 3 to 6 hr after spawning exhibit lower food conversion efficiencies relative to controls developed from eggs which remained in the spawning salinity. He does not, however, compare food conversion efficiencies of fish acclimated to a common salinity which had developed at different salinities. It is not clear, therefore, whether the observed differences in food conversion efficiencies are reversible upon acclimation to common conditions. Bradley (1978) showed that copepods raised at 20° C are more tolerant of high temperatures than those raised at 10° C. However, these results could have arisen from selection during rearing or to a thermal acclimation that requires more than two days' exposure to a common

temperature to disappear. Schneider (1968) concluded that differences in acclimated oxygen uptake rates among populations of the crab *Rhithropanopeus harrisi* can be reversed only in part by breeding the crabs at a common temperature, but the full report of these data has not appeared.

The mode of reproduction in the actinian *Haliplanella luciae* (Verrill) offers an interesting approach to the question of nongenetic adaptation which is irreversible within a period of prolonged acclimation to common conditions. This species reproduces largely by asexual mechanisms, longitudinal fission being the most common (Shick and Lamb, 1977). The fission process in *Haliplanella luciae* and the effects of temperature and other environmental factors on it have been studied in detail by several authors (Torey and Mery, 1904; Davis, 1919; Uchida, 1932; Miyawaki, 1952; Minasian, 1976). Longitudinal fission can be induced in the laboratory by storing the animal at 10° C or below for 6 to 8 weeks, followed by raising the temperature above 10° C (Sassaman, personal communication). This treatment apparently simulates increasing water temperatures during spring. After fission the progeny regenerate tissues to close the body wall torn in the process. Thus isogenic clones of animals can be raised under different environmental conditions and later compared, virtually eliminating the possibility of genetic variability among the experimental animals.

In the present study, three generations of isogenic anemones were reared at two temperatures, and their rates of oxygen uptake compared after acclimation to common thermal conditions. A few observations were also made on the electrophoretic banding patterns of five enzymes and on the dimensions of the gas exchange surface.

Materials and Methods

Collection, maintenance of animals and experimental design

Specimens of *Haliplanella luciae* (Verrill) were collected from the York River estuary (15 to 21‰) at the mouth of Indian Field Creek, Virginia in January 1977 (10° C). Included in this sample were animals of Uchida's (1932) color types 1 and 3, indicating genetic heterogeneity in the population. Assuming all animals to be genetically unique, single individuals were placed in 30-ml plastic beakers filled with York River estuary water. One half of the animals were placed at 28° C and the remainder stored at 18° C. After approximately 2 weeks the animals used at both temperatures had undergone fission, each original individual producing two or more progeny (clonemates). When regeneration was complete, the animals were transferred to 5° C for 6 to 8 weeks. At the end of this period, the clonemates were separated and allowed to undergo fission and subsequent formation of new tissue at different developmental temperatures: one member of each clone was randomly chosen and placed at 18° C and its clonemate was placed at 28° C. After fission and regeneration, the second generation clonemates were separated, stored at 5° C for 6 to 8 weeks and then returned to their developmental temperature, where a third generation was produced. Thus there are four groups of animals according to developmental temperature and the number of generations produced at a given temperature: two consist of animals taken from the field that

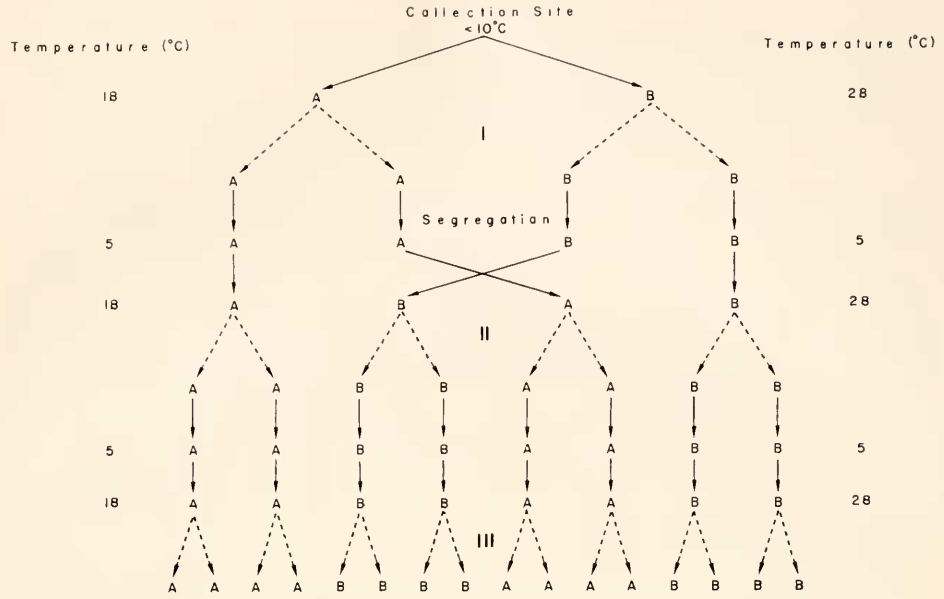

FIGURE 1. Cloning of *H. luciae* at 18° and 28° C. The example shows two animals (A and B) assumed to be genetically unique. Solid arrows indicate transfer from one temperature to another; dotted arrows indicate fission and regeneration. Roman numerals indicate generation.

divided and formed new tissue three times at either 18° or 28° C; one consists of animals that divided first at 18° C and a second and third time at 28° C, and one of the animals that divided a second and third time at 18° C. Because of the difference in the number of fissions at each developmental temperature (Fig. 1), a control was necessary to permit pooling the data for all clones at a particular developmental temperature. The oxygen uptake rates for animals which divided twice at a developmental temperature were compared with those for animals which had divided three times at that developmental temperature. The important aspect of the experimental design is, however, that each individual has at least one clonemate treated in the same way but given the alternative thermal regime (Fig. 1), and that each of the four experimental groups contains members of the same clones, in the same proportions. Thus the rearing procedures cannot have permitted selection.

Development and growth occurred at the specified temperature in York River estuary water (15 to 21‰, with members of all groups given the same salinity regime). At least 4 days prior to the measurement of oxygen uptake, the salinity was brought to 18‰. Animals were fed an excess of freshly hatched *Artemia* nauplii every other day.

Oxygen uptake measurements

Oxygen uptake ($\dot{V}_{O_2}$) of the third generation clonemates was measured at the developmental temperature (TD), the same as the acclimation temperature

(TA). These data are designated TD = 18° C = TA and TD = 28° C = TA. Other, but isogenic, individuals were transferred to the alternative temperature, held there for 2 to 4 weeks and oxygen uptake measured. These data are designated TD = 18° C, TA = 28° C, and TD = 28° C, TA = 18° C. All measurements were made in millipore filtered water at 18‰ salinity by adjusting the concentration of natural York River estuary water (15 to 21‰) with distilled water or commercial sea salt (Dayno Corp). Animals were starved for 60 to 84 hr before an experiment, and transferred to the respirometry chambers the night before the measurement to permit attachment to the wall of the chamber. Oxygen depletion was measured with a Yellow Springs Instrument Co. Model 53 Biological Oxygen Monitor, using a high sensitivity (0.0005 in) teflon membrane and a 1 × 10 mm stirring bar. The animals were permitted to adjust to stirring for 1 hr before sealing the chamber and the data were discarded when the tentacles were retracted before completion of the measurement. All data in the P_{O_2} interval 110 to 159 mm Hg were analyzed. For each experiment a measurement of the oxygen uptake of the electrode was made using an empty chamber, and this value was subtracted from the experimental rate.

At the end of an experiment animals were stimulated to expel fluid from the gastrovascular cavity, removed from the chambers, lightly blotted and weighed to the nearest tenth of a milligram (Cahn Electrobalance, Model G).

Electrophoresis

Animals were starved for one week and then frozen whole, either singly or in groups from the same clone and treatment group, in two volumes of buffer: 0.05 M tris (titrated to pH 8 with concentrated HCl) and 0.001 M EDTA to which NADP (4 mg/liter) was added. Freezing took place quickly in a bath of dry ice and acetone, and the samples were then stored at −70° C until homogenized. Samples were thawed, homogenized and centrifuged. When necessary the supernatants were diluted as much as 50% with distilled water to obtain 25 μl which were loaded into slots of horizontal starch gels (13% w/v; Sigma starch).

Gel and electrode buffers for glucose-6-phosphate dehydrogenase (G-6-PDH, E.C. 1.1.1.49) and isocitrate dehydrogenase (IDH, E.C. 1.1.1.42) were modified from Markert and Faulhaber (1965): 0.9 M tris, 0.5 M boric acid, 0.02 M EDTA diluted 1:20 for the gel buffer and 1:6 for the electrodes; in addition, 30 mg and 10 mg NADP were added to the gel and to the cathodal buffer tray, respectively. The gel and electrode buffers for malate dehydrogenase (MDH, E.C. 1.1.1.37) were those described by Nichols and Ruddle (1973). The discontinuous LiOH buffer system of Selander, Hunt, and Yang (1969) was used for phosphoglucose isomerase (PGI, E.C. 5.3.1.9) and hexokinase (HK, E.C. 2.7.1.1.). The period of electrophoresis was 3 to 4 hr. After electrophoresis at 350 volts, gels were sliced and stained with slight modifications of the procedures described by Brewer (1970) and Shaw and Prasad (1970). The gels were preserved overnight in 50% ethyl alcohol, blotted and stored in plastic wrap until photographed.

Gas exchange surface area

Animals from the TD = 18° C = TA and the TD = 28° C = TA treatment groups were anesthetized with $MgCl_2$. Tentacles were counted and the external

surface areas of the body wall and the tentacles were approximated from measurements made at 10 to 25×, using formulas for the frustum of a cone and for a cylinder, respectively.

Statistical analyses

Mean $\dot{V}_{O_2}$ values were computed by determining the rate during each time interval of the continuous record of the change in oxygen concentration. Thus, unless specifically stated otherwise, the N value given in the results represents the sum of all data obtained from the members of an experimental group, which are treated as a homogenous population.

The comparison was complicated, however, by the different body sizes of the experimental groups (see below). Two alternative procedures were used to eliminate differences due to body size from the conclusions. First, a regression coefficient of −0.43 was used to correct rates to a common weight of 1.6 mg, a representative value, by covariance analysis. This coefficient was determined by performing a regression analysis on data from the TD = 18° C = TA treatment group ($n = 14$ animals, $r = 0.75$, $P < 0.001$). This relationship is homogenous, showing no evidence of the discontinuity observed by Shick, Brown, Dolliver, and Kayar (1978). Second, the data were grouped by weight classes established from an analysis of frequency distributions, and the comparison made within the classes.

Although the experimental design permits analysis of the data as paired observations, the available procedures for computing Student's t require an equal number of observations in each member of the pair, a condition which was not met. The element of genetic homogeneity was maintained in the experimental design by keeping virtually constant the percent of the total number of observations made on each clone under each combination of developmental and experimental temperatures.

Results

Effect of developmental temperature on body size

At 28° C fission occurred more rapidly and more frequently than at 18° C, resulting in more numerous but smaller progeny (see below). As suggested earlier by a number of investigators (Ray, 1960; Mangum, 1963; Shick, 1972) this finding suggests that the often observed phenomenon of a larger body size in the colder regions of a species range results from latitudinal differences in developmental temperature.

The frequency distribution of weight in each experimental group (Fig. 2) indicates, in general, that body size of the animals is more widely distributed in the 18° C developmental group than in the 28° C developmental group. Almost 73% of the animals in the TD = 28° C = TA treatment group weigh 1.1 mg or less, and 91% of the animals in the TD = 28° C, TA = 18° C experimental group weigh 2.0 mg or less. In contrast, only 29% of the animals in the TD = 18° C = TA group and 17% of those in the TD = 18° C, TA = 28° C treatment group weigh 1.1 mg or less. In these groups, reared at low temperature, greater frequencies of animals in the higher weight classes are also evident (Fig. 2). A

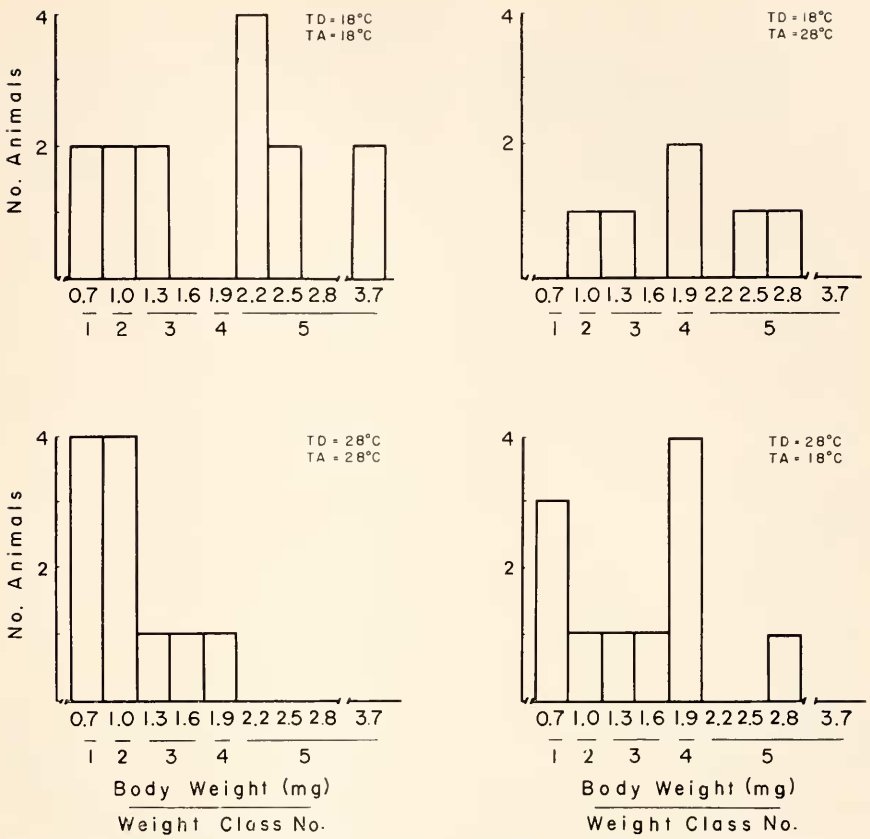

FIGURE 2. Frequency distribution of body weight in treatment groups, designated by developmental (TD) and acclimation (TA) temperature.

comparison of the frequency distributions by weight class of the two developmental temperature groups emphasizes the trends (Fig. 3). The mean body weight of the TD = 28° C = TA and TD = 28° C, TA = 18° C animals is 1.4 mg; the mean weight of the two TD = 18° C groups is 2.0 mg. The median weight class of the two TD = 28° C groups is 0.6 to 0.8 mg, and the corresponding figure for the TD = 18° C groups is 2.1 to 2.3 mg.

Oxygen uptake

Three clones of animals which first divided at 18° C and one clone of animals which first divided at 28° C were used to measure O_2 uptake; all clones produced third generation animals at both developmental temperatures. No significant differences were found between the control groups ($P > 0.85$) indicating that V_{O_2} in clones that have produced two generations at a particular developmental temperature is the same as in clones that have produced three generations at that developmental temperature.

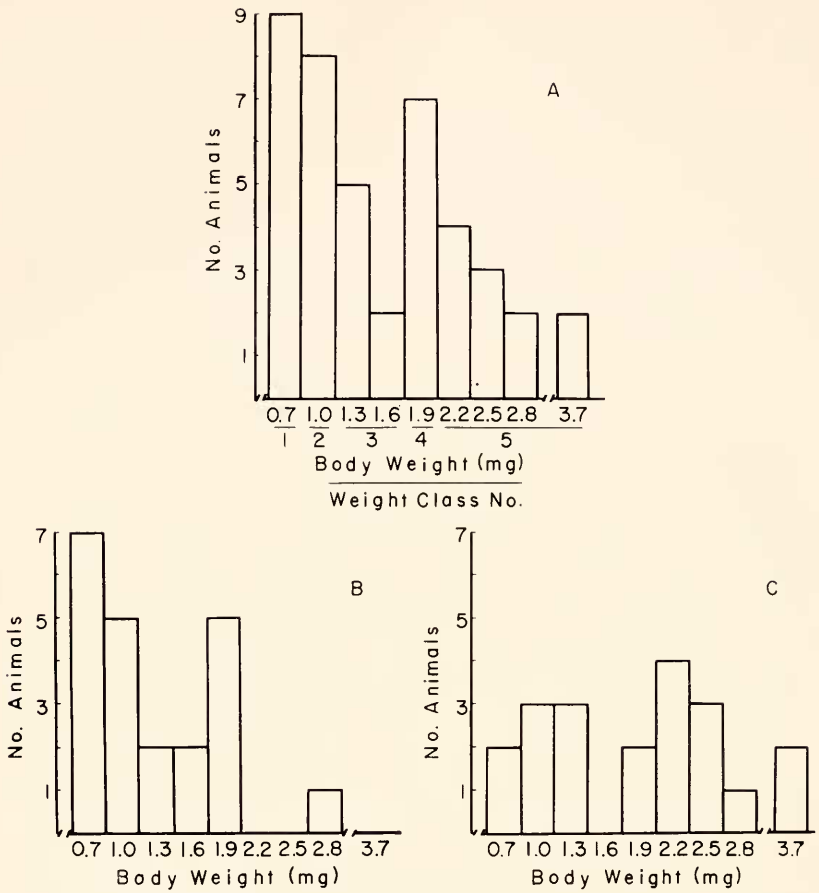

FIGURE 3. Frequency distribution of body weight in (A) all animals used in the oxygen uptake experiment, (B) TD = 28° C developmental groups only, and (C) TD = 18° C developmental groups only.

The mean weight corrected oxygen uptake rates of the two TD = 18° C groups are significantly higher ($P < 0.01$) than those of the two TD = 28° C groups at both acclimation temperatures (Table I). At the test temperature of 18° C the rates in the TD = 18° C groups are 18% higher than those of the TD = 28° C groups, and at 28° C the rates are 19% higher. However, using the raw data for the smaller animals produced at 28° C and the larger ones produced at 18° C, the difference diminished to 4% at the test temperature of 18° C and it is actually reversed at 28° C (−8%). Thus a confounding influence of developmental temperature, its effect on body size, may obscure the direct effect on the acclimated rate of oxygen uptake.

The frequency distribution by weight of all animals used in the oxygen uptake experiments is shown in Figure 3. When the rates are compared by weight class, significant differences between the TD = 18° C and the TD = 28° C groups are

TABLE I

A. *Oxygen uptake (μl/g·hr) in isogenic specimens of* Haliplanella luciae *reared for two to three generations at different temperatures. Mean ± s.e. (N). Probability values from one-way analyses of variance comparing developmental temperature groups at each test (= acclimation) temperature.*

Test temperature (°C)	Body wt. (mg)	Developmental temperature (°C)		*P*
		18	28	
18	1.9 ± 0.2	243.7 ± 12.0 (111)	235.0 ± 16.2 (72)	n.s.
28	1.3 ± 0.1	487.1 ± 24.1 (36)	524.7 ± 21.6 (54)	n.s.

B. *Oxygen uptake (μl/g·hr) corrected to a common body weight (1.6 mg) by covariance. Data analysis as above.*

Test temperature (°C)	Developmental temperature (°C)				*P*
	18	Q_{10}	28	Q_{10}	
18	257.0 ± 9.4 (111)	1.99	218.7 ± 13.9 (72)	1.96	$P < 0.001$
28	510.3 ± 22.6 (36)		427.8 ± 14.5 (54)		0.0018

found in one of the four possible comparisons at 18° C and in two of three at 28° C (Table II). The trend of higher rates in the TD = 18° C groups is uniformly consistent, but the probability levels rise due to the smaller number of observations (N).

TABLE II

Weight corrected oxygen uptake rates (μl/g·hr), Mean ± s.e. (N). Probability (P) values from one-way analyses of variance comparing the developmental temperature group rates at each experimental (= acclimation) temperature for each weight class.

Experimental temperature (°C)	Weight class		Developmental temperature (°C)		*P*
	No.	Range (mg)	18	28	
18	1	(0.6–0.8)	270.5 ± 46.2 (10)	194.5 ± 33.2 (19)	n.s.
	2	(0.9–1.1)	269.8 ± 38.0 (11)	146.6 ± 41.4 (7)	0.0499
	3	(1.2–1.7)	218.6 ± 22.7 (23)	159.1 ± 21.8 (16)	n.s.
	4	(1.8–2.0)	—	288.5 ± 18.7 (24)	—
	5	(2.1–3.7)	254.7 ± 11.2 (67)	258.8 ± 4.1 (6)	n.s.
28	1	(0.6–0.8)	—	444.0 ± 22.8 (20)	—
	2	(0.9–1.1)	413.3 ± 38.1 (6)	417.4 ± 27.3 (20)	n.s.
	3	(1.2–1.7)	568.9 ± 13.8 (5)	408.3 ± 37.7 (9)	0.003*
	4	(1.8–2.0)	608.9 ± 34.3 (13)	439.9 ± 24.1 (5)	0.01
	5	(2.1–3.7)	427.4 ± 31.6 (12)	—	—

* Bartlett's Test indicates non-homogenous variances for the two developmental groups of this weight class. A Separate Variance Estimate *t*-test was used to make this comparison.

Table III

Gas exchange surface area (cm^2/g wet wt.) in each developmental temperature group of Haliplanella luciae. *N = Number of animals. Weight classes as in Table II.*

Developmental temperature (°C)	Weight class	N	No. tentacles	Tentacle surface area	
				cm^2/g	% Total body surface area
18	1	2	13	5.6	33.9
	2	2	16	5.0	57.5
	3	3	20	7.6	42.7
28	1	2	18	19.2	73.5
	2	4	30	14.7	72.9
	3	3	29	18.6	78.7

Q_{10} values for both developmental temperature groups (Table II) are similar, and they also approximate the values reported previously over the same temperature range (Sassaman and Mangum, 1970). Thus, no evidence of a change in temperature sensitivity with developmental temperature is seen. No overall pattern of temperature sensitivity is apparent in the data grouped by weight class, thus supporting previous conclusions that it is not correlated with body size (Sassaman and Mangum, 1970). Nor is there a trend in Q_{10} with developmental temperature.

Gas exchange surface

A possible explanation of the irreversible difference between cold and warm-reared anemones is a change in gas exchange surface area. This hypothesis would be supported by an increase in tentacular surface, the primary site of oxygen uptake in epifaunal anemones (Sassaman and Mangum, 1972; Shick *et al.*, 1978) in the TD = 18° C groups. In fact, measurements of gas exchange surface (Table III) indicate the opposite relationship, suggesting that the difference would be even larger if the gas exchange surface had remained the same.

Surface area is not correlated with body size. Pooling data for all weight classes at each developmental temperature, anemones in the TD = 28° C groups have more tentacles and a significantly larger (Mann-Whitney U-Test) total surface area available for gas exchange than anemones in the TD = 18° C groups. Not only are the tentacle numbers greater in the TD = 28° C groups, but the average surface area per tentacle is greater as well. Moreover, the percent of the total body surface area contributed by the tentacles always exceeds 73 in the TD = 28° C groups, compared to a maximum of 57.5% in the TD = 18° C animals (weight class 2).

Electrophoresis

The enzymes examined showed no qualitative differences in banding pattern either among the separate clones of one treatment group or among clonemates of different treatment groups. The results for all of the enzymes tested are similar

and they suggest that the population of *H. luciae* at Indian Field Creek exhibits no variation at these loci. Of the five enzyme systems examined, only IDH was clearly polymorphic, the banding pattern suggesting fixed homozygosity at two loci. However, no qualitative differences in banding pattern were detected, either between clones or within clones. Since these loci differ in latitudinally separated populations and within populations of *H. luciae* (Shick and Lamb, 1977), the uniform pattern could reflect conservative selection pressures on these loci for this particular population. Regardless, there is no evidence that the different alleles were either induced or repressed by the formation of tissues at different temperatures.

Discussion

Differences in aerobic metabolism that cannot be reversed by 2 to 4 weeks acclimation to common conditions clearly result from reproduction, regeneration and the formation of new tissues at different temperatures, when the effects of other variables are eliminated from the data. The direction of the change is generally compensatory, resulting in higher metabolic rates in the animals produced at low temperatures. The magnitude of the change, however, is not great enough to override opposing effects of developmental temperature on body size and gas exchange surface area. Oxygen uptake rates go up at low developmental temperature but down with increasing body size and decreasing tentacular surface, and body size increases and tentacular surface area decreases at low developmental temperature. The net outcome of the concomitant and counteracting trends is little or no difference between the two groups, when body size and gas exchange surface are retained as variables.

The question remains of the origin of the metabolic difference in animals of the same size. One alternative is a direct but irreversible effect of developmental temperature on the metabolic machinery within the cell. The five enzyme systems examined were selected in part for their variability in the species (and in other anemones; Manwell and Baker, 1970), and in part for their relation to aerobic metabolism. Hexokinase, phosphoglucose isomerase and isocitrate dehydrogenase vary in different populations of *H. luciae* (Shick and Lamb, 1977); isocitrate dehydrogenase and malate dehydrogenase catalyze reactions in the tricarboxylic acid cycle and glucose-6-phosphate dehydrogenase regulates a branchpoint of the pentose phosphate pathway. No qualitative changes with developmental temperature or acclimation temperature were found for any of the five enzymes tested. Quantitative data, on various enzyme systems, however, are not available. Robert and Gray (1972) have shown an increase in the specific activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the blue crab *Callinectes sapidus* during cold exposure, accompanied by no qualitative electrophoretic changes. A thorough test of this possibility, which we regard as a promising hypothesis, would entail an exhaustive investigation of polymorphic enzyme systems that influence the rate of oxidative reactions, directly or indirectly.

The only obvious alternative explanation would be an irreversible effect of developmental temperature on the gas exchange system. Since fluid movements on both sides of the tentacles are generated by cilia, an irreversible difference in oxygen convection would seem to be highly unlikely. An irreversible change in gas

exchange surface was detected, but its effect on oxygen uptake should be the opposite of that observed. Thus a systemic explanation seems highly unlikely.

Regardless of its basis, the present findings clearly demonstrate the reality of irreversible physiological adaptation, and they raise the possibility that the compensatory responses found earlier in geographically separate populations of the species may result from developmental as well as acclimation temperature.

We are grateful to R. J. Hoffmann for the use of his facilities and expertise in performing the electrophoreses, and to D. Reed for his help with data analysis.

Summary

1. Isogenic clones of *H. luciae* were raised at each of two developmental temperatures, 18° and 28° C. Despite prolonged acclimation to common thermal conditions, oxygen uptake rates differ according to the temperature of reproduction, regeneration and development.

2. The effects of developmental temperature, however, are masked by body size differences. Only when this variable is eliminated can the underlying effect of developmental temperature be detected.

3. The irreversible change is not due to an increase in the gas exchange surface area at the primary site of O_2 uptake, the tentacles.

4. No qualitative changes in banding patterns for five enzymes (HK, PGI, IDH, MDH, G-6-PDH) were found.

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